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INTERACTIONS BETWEEN
IMMUNOCOMPETENT CELLS AND
TARGET CELLS OR ANTIGENS*

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As the interaction of immunocompetent cells with target cells or antigen is a broad subject, this brief review is limited to certain *in vitro* studies of the problem. The first part will be devoted to the destruction of target cells or tissues by the addition of immunocompetent lymphoid cells. The second part will deal with the stimulation and blast formation of lymphocytes by phytohemagglutinin and antigen, and the third with the inhibition of cell migration by antigen. As the symposium is concerned with cellular factors in autoimmunity, I shall try to discuss these three parameters of cellular behavior as they relate to one model of autoimmune disease, namely experimental allergic encephalomyelitis.

In models of target cell destruction by sensitive cells, such as those

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of Rosenau and Moon,^{1, 2} Wilson,³ and others, animals of one strain are sensitized with tissues from another strain. Lymphoid cells from the sensitized animals are placed on top of monolayers of target cells obtained from the same strain as that used to sensitize. Using this type of experiment, many workers have obtained similar results. The lymphoid cells from sensitive animals, but not those from normal controls, first aggregate about the target cells, a process that usually takes several hours. Cytotoxic changes are then observed after 12 hours; they reach their peak at 24 to 48 hours. The reaction occurs without the addition of complement, and thus far no complement has been detected in lysates of the sensitive cells (although the test used, the lysis of erythrocytes, would require the complete complement system and would not detect the presence of a few components).³ In these studies it is necessary that the sensitive cells be alive, otherwise the effect is not produced. Wilson was able to suppress the cytotoxic action of sensitive cells with Imuran, using doses that inhibited certain metabolic activities of the lymphocytes without killing them.⁴ Wilson's quantitative studies suggest that only 1 to 2 per cent of the lymphocytes actually lyse target cells. By using irradiated target cells, he also showed that the sensitive lymphoid cells were not just preventing target cell growth. Although Rosenau and Moon demonstrated that the reaction they were observing was immunologically specific,⁵ most workers have compared the effect of sensitized cells with normal, unsensitized cells and not with the effect of cells sensitized to an unrelated antigen. Further, a few workers have been able to simulate the toxic effect of sensitive cells by adding large quantities of normal cells. Recent studies suggest that such sensitivity can be transferred to normal lymphocytes with RNA⁶ or ribosomes⁷ from sensitive lymphoid cells.

Most investigators believe that the sensitive lymphoid cells, in order to exert cytotoxic action, must be in intimate contact with the target cell. This is based on experiments such as those of Rosenau and Moon, and of Wilson, all using millipore chambers. When sensitive lymphoid cells were separated from the target cells by a millipore filter, there was no longer any destruction of the target cells. One objection may be raised to the conclusions drawn from these experiments. Contact between sensitive cells and target cells at some time may be necessary; however, these experiments do not rule out the possible mediation of cell destruction by a soluble material. It would, for example, be of

great interest to determine whether lymphoid cells are stimulated by target cells on the *same* side of a millipore chamber to produce material that would be cytotoxic for the target cells on the other side.

In many but not all of the studies on target cell destruction, it was also found that sera from sensitized animals were cytotoxic for the target cells.² This reaction, however, was found to be complement-dependent and to occur more rapidly, usually in one hour. Except for one experiment, to be discussed later, such sera were not able to sensitize normal lymphoid cells to lyse target cells. Indeed, in experiments such as Moller's, sera from sensitized animals inhibited the cytotoxicity of sensitized cells.⁸

Similar studies have been applied to experimental allergic encephalomyelitis (AE). Bornstein and Appel showed that sera from animals with AE were capable of demyelinating cultures of rat cerebellum.^{9, 10} The effective material in the sera was γ -globulin, and the reaction was complement-dependent and reversible. Of interest is the finding by these workers that sera from patients with multiple sclerosis as well as certain other neurologic diseases also demyelinated brain cultures.¹¹ Lamoureux *et al.* have found recently that cerebrospinal fluid from monkeys with AE as well as from patients with multiple sclerosis was capable of demyelinating cultures of brain tissue.^{12, 13} It should be noted that sera from two monkeys that were sensitized with brain but did not develop the disease inhibited the cytotoxic action of sera from monkeys with the disease.¹² This is reminiscent of the finding of Paterson that certain antibodies from animals with AE will actually protect against the disease.¹⁴ The presence of a cytotoxic material in cerebrospinal fluid from other neurologic diseases, especially in certain viral diseases such as infection with Coxsackie B₂ virus, may limit to some extent the primary etiological role of such factors.¹³

Studies by Koprowski and Fernandes and by Winkler demonstrated that sensitive cells from animals with AE can kill glial cells and demyelinate cultures of nervous tissue.^{15, 16} Winkler found that lymphoid cells could do this at a time when sera did not.¹⁶ Winkler and Arnason have further investigated the mechanism of this reaction. Since it was known that thymectomized animals as well as patients with ataxia telangiectasia had decreased delayed hypersensitivity and showed decreased IgA in their serum, these workers attempted to block the action of sensitized cells with antibody to IgA. They found that the demyelination of rat

trigeminal ganglion by sensitive rat lymphoid cells was blocked when rabbit antibody to rat IgA was added to the cultures, but not when rabbit antibody to rat IgM or IgG was added.¹⁷ This suggested the possibility, among others, that lymphocytes might act either by secreting or having on their surfaces some material similar to IgA. The unique finding in Koprowski's study was the observation that serum from sensitized animals, while not toxic itself, would sensitize lymphoid cells obtained from animals sensitized with complete Freund's adjuvant, but without brain antigen. The possibility that in this case antibody sticks the lymphoid cells to the target tissue and that then the lymphoid cells kill the target cells leads to another group of experiments concerning this *in vitro* model.

Briefly, Holm and Perlmann¹⁸ and Moller¹⁹ showed that normal lymphoid cells were cytotoxic to allogenic cells if they were stuck to these by phytohemagglutinin or by antisera to the lymphoid cells. This cytotoxicity occurred only when lymphoid cells and target cells were antigenically histoincompatible. However, Moller found that cells from F₁ hybrids, which should not be able to recognize parental cells as foreign, nevertheless were capable of killing target cells of parental origin. Such lymphoid cells were active after they had been irradiated. Moller has suggested that this reaction is due to differences in cell surface structure, possibly unrelated to histocompatible antigens. The recent observation by Moller that in human cell systems it is possible to observe cytotoxicity with autologous cells on autologous targets complicates the interpretation of this phenomenon.²⁰ It should be recalled that both phytohemagglutinin and antibody do more than glue the lymphoid cell to the target. Both these materials are capable of stimulating and transforming lymphocytes.^{21, 22} Further, Rosenau has reported that, if lymphoid cells from a nonsensitive animal were stuck to allogenic target cells with polylysine, they did not cause any target cell damage.² Thus the reaction involving "normal" cells with phytohemagglutinin or antiserum may not simply occur with normal cells but with cells that have been artificially stimulated to differentiate; this phenomenon may be different from the reaction of specifically sensitive cells with target cells that was discussed earlier.

Do the sensitive lymphocytes die in the cytotoxic process? Rosenau, who observed cultures with cinematography, thought they did.² However, in most systems this has not been specifically studied. It would be

quite interesting, for instance, to see whether thymidine-labeled sensitive lymphocytes released label into the media after contact with target cells. Before leaving the subject of target cell reactions, it should be pointed out that macrophages have also been shown to exert cytotoxic effects on target cells.^{23, 24}

The second model to be discussed, the stimulation of lymphocytes by antigen, is proving to be one of the most useful tools available for the study of immune cells in man. The early findings of Nowell²¹ on the transformation of lymphocytes by phytohemagglutinin, and those of Permain *et al.*²⁵ and Hirschhorn *et al.*²⁶ on the stimulation of these cells by specific antigen have led to many interesting clinical observations. It has been shown, for instance, that anergic patients, such as those with Hodgkin's disease and sarcoidosis, have lymphocytes that respond poorly to phytohemagglutinin and other antigens.^{27, 28} In this context, it is of note that Holm and Perlmann reported that lymphocytes from patients with Hodgkin's disease were less cytotoxic to allogenic target cells when stuck to these with phytohemagglutinin than were normal lymphocytes.²⁹ These workers also observed that this finding correlated with lack of transformation of the cells by phytohemagglutinin. Smithwick and Berkovich recently found that lymphocytes from tuberculin-positive patients were unresponsive to tuberculin during measles, and that measles virus, when added directly to PPD-sensitive lymphocytes, depressed stimulation by PPD (but not, interestingly enough, stimulation by phytohemagglutinin), suggesting a mechanism for the anergy often observed during the course of measles.³⁰ Of special importance is the recent finding by Fowler, Morris, and Whitley that lymphocytes from six patients with multiple sclerosis were stimulated to transform by autologous or homologous cerebrospinal fluid, whereas cells from nine control patients were not.³¹ It will certainly be of great interest if such cells are also found in patients with viral encephalitis or if the phenomenon is specific for multiple sclerosis.

The exact relation of lymphocyte stimulation to delayed hypersensitivity is still not completely understood, and further study is necessary before we can say without question that it is a specific manifestation of delayed hypersensitivity.

The third model, inhibition of cell migration by antigen, first described by Rich and Lewis,³² is one we have been studying during

the past few years in the hope of learning more about sensitive cells.³³ The technique, based on the method of George and Vaughan,³⁴ consists, first, of placing peritoneal exudate cells obtained from animals with delayed hypersensitivity in capillary tubes. The cells then migrate out of the tubes onto glass coverslips in culture chambers. This migration is inhibited when specific antigen is in the media, and such inhibition can easily be quantitated. The reaction is immunologically specific. Furthermore, cells from animals making antibody but not exhibiting delayed hypersensitivity are *not* inhibited by antigen, and normal cells are not sensitized by incubation in sera from sensitized animals.³³

Since this phenomenon appeared specific for delayed hypersensitivity, a study was carried out in collaboration with Dr. Philip Paterson to determine whether guinea pigs with AE might yield cells specifically sensitive to the encephalitogenic antigen. We found that peritoneal cells from guinea pigs with AE were indeed inhibited by extracts of guinea pig brain.³⁵ This appeared to be an organ-specific reaction. Guinea pig kidney extract, for example, did not inhibit the migration of these cells. Reports that neonatal brain does not produce either clinical or histologic evidence of AE and thus lacks the encephalitogenic antigen were confirmed in our experiments by means of neonatal rat brain. The absence of the encephalitogenic antigen in neonatal brain made it possible to determine whether cellular sensitivity in AE is directed specifically to the encephalitogenic antigens and not to other components in brain unrelated to the disease. It was found that neonatal rat brain did not inhibit the migration of cells obtained from guinea pigs with AE. In contrast, adult rat brain and guinea pig brain, which readily produce the disease, did inhibit the migration of such cells. These findings indicate that the cellular sensitivity is directed against a component that appears in the maturing nervous tissues parallel with the appearance of the encephalitogenic antigen—if indeed it is not the encephalitogenic antigen itself. The finding that cells from animals with AE are specifically sensitive to the antigens known to produce AE suggested strongly that cells of such specific sensitivity might well play an important role in the pathogenesis of the disease.

In light of the findings of Koprowski, we also tried to see whether sera from guinea pigs with AE, whose own cells had been inhibited by brain extract, could confer such sensitivity upon cells from animals sensitized with complete Freund's adjuvant but without brain. These

experiments were uniformly negative. We were unable to sensitize such cells with sera.

Further studies on the inhibition of cell migration have shed some light on its mechanism, and some conclusions from these studies may be extended to the general problem of delayed hypersensitivity. Early in the course of our studies we found that only a few sensitive cells need be present for the whole population to be inhibited by antigen. By mixing cells from sensitive animals with cells from normal animals, we found that as few as 2.5 per cent of cells need come from the sensitive animals for inhibition to be observed.³⁶ The sensitive cells had to be alive for this to take place. Puromycin prevented the reaction.³⁷ These findings are consistent with the studies of McCluskey *et al.* and others who showed that most of the cells at the skin test site following passive transfer of delayed hypersensitivity were nonsensitive host cells.³⁸

In an attempt to determine what cell type was the sensitive cell, we obtained cell preparations from lymph nodes of highly sensitive animals; these preparations contained about 95 per cent lymphocytes. Such cells were *not* inhibited by specific antigen, but when they were added in small numbers to normal peritoneal exudate cells, the resulting population *was* inhibited by antigen.³⁹ Bloom and Bennett demonstrated that sensitive peritoneal cells freed of lymphocytes were no longer inhibited by PPD, while the lymphocytes, when added to normal peritoneal cells, would cause inhibition by antigen.⁴⁰ It has been shown by Bloom and Bennett and by results from our laboratory that the lymphocytes act by producing a soluble material that inhibits the migration of normal peritoneal cells, presumably the macrophages. For instance, if lymphocytes from a guinea pig sensitive to ovalbumin are incubated for 24 hours with ovalbumin, the cell-free supernatant, when added to normal cells, inhibits cell migration. These same lymphocytes, when incubated in antigen-free medium or with an unrelated antigen such as bovine γ -globulin, do not form the active material. If puromycin is added to the mixture of sensitive lymphoid cells and antigen, the lymphoid cells no longer yield an active supernatant. The active substance is macromolecular, i.e., it is nondialyzable; it is heat-stable to 56° C. for 30 minutes.³⁶ We are presently attempting to characterize the material. The data available suggest an explanation for the phenomenon of inhibition of cell migration by antigen. The first event may be the reaction of specific sensitive lymphocytes with antigen.

This interaction results in the production of substances that either alone or in combination with antigen affect the remaining nonsensitive cells, presumably macrophages. The detection of material with these biological properties raises interesting questions as to its nature: whether continued presence of antigen is required for its effect, its relation to known immunoglobulins, and its relevance to *in vivo* events of delayed hypersensitivity.

Each of the three models discussed measures a different parameter of lymphocyte behavior in response to antigens. The first demonstrates the cytotoxic action of sensitive lymphocytes on target cells and may yield valuable information on the exact mechanism by which lymphocytes destroy target cells. This model may be directly related to the role that such cells play in homograft rejection, to tissue destruction by lymphocytes in certain autoimmune diseases, and to tumor immunity. The second model, the transformation of lymphocytes by antigen, focuses directly on the lymphocyte itself. How this transformation affects the function of the lymphocyte is not yet known. Although this phenomenon appears to be an immune reaction, the relation of lymphocyte transformation to antibody formation and delayed hypersensitivity remains to be determined. The third system described measures the presence of cells reflecting delayed hypersensitivity. It is presently of use in characterizing the soluble material which sensitive lymphocytes produce when stimulated by antigen. This material, *in vitro*, affects the behavior of nonsensitive macrophages, and may play a similar role *in vivo*. Such a material may also be involved in target cell reactions. It is not known at present whether these different models are measuring different functional parameters of the same cell, i.e., whether the same lymphocyte may be stimulated to transform, or make a soluble material that can affect normal macrophages, or can be cytotoxic when specifically sensitive to a target cell. It is possible that different groups of lymphocytes are involved in each of these reactions. It has been suggested that lymphocytes should not be considered as one type of cell; indeed, it appears that they may be a group of cells with differing life spans and differing functions.^{41, 42} It remains to be determined in what manner these various models involving lymphocytes are related to each other.

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